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Spore Germination and Early Gametophyte Development in *Stromatopteris*

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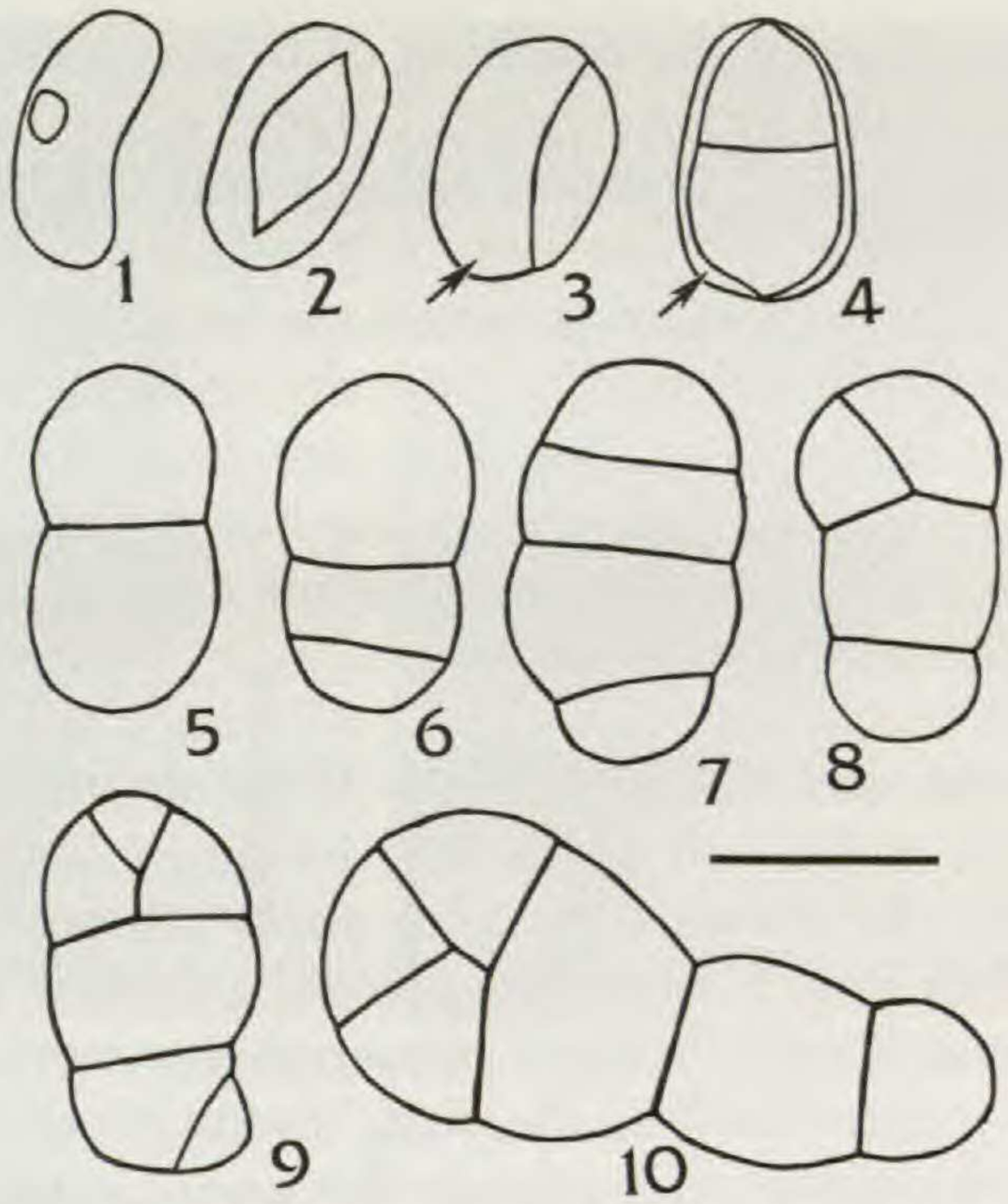
ABSTRACT.—Spores of *Stromatopteris moniliformis* (Gleicheniaceae) germinated after three months in the dark on a nutrient medium containing minerals and 0.5% glucose. Small cylindrical gametophytes with apical cells and septate rhizoids were grown under these conditions. The pattern of cell divisions for early gametophyte development is that of the Gleicheniaceae.

Spores from species of eusporangiate pteridophytes with mycorrhizal gametophytes germinate in the dark (Whittier, 1973, 1981, 1983; Gifford and Brandon, 1978; Whittier and Braggins, 1994). The dark-germination of spores from the Ophioglossaceae, Psilotaceae, and those taxa of the Lycopodiaceae with subterranean gametophytes insures that the mycorrhizal gametophytes will develop in the soil. This study was undertaken to determine if the dark-germination of spores from species with mycorrhizal gametophytes is a more general phenomenon in the pteridophytes. *Stromatopteris* spores were used to investigate if spores from a leptosporangiate species with subterranean gametophytes (Bierhorst, 1968) will germinate and initiate gametophyte development in the dark. Also, it would be of interest to know if the early developmental stages of *Stromatopteris* gametophytes have any similarity to those stages of the eusporangiate pteridophytes.

MATERIALS AND METHODS

Spores of *Stromatopteris moniliformis* Mett. were obtained from plants in New Caledonia and were sown within three weeks of their collection. The spores were surface-sterilized with 20% Clorox by the method of Whittier (1964), suspended in sterile water, and sown on 15 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were then tightened. Most of the cultures were maintained at 21±1°C in the dark, but a few were exposed to a daily 12 hour photoperiod (50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) from Gro-lux fluorescent lamps.

The nutrient medium contained 100 mg NH_4Cl, 100 mg $MgSO_4 \cdot 7H_2O$, 40 mg $CaCl_2$, and 100 mg K_2HPO_4 as a final concentration per liter. In addition, 4 ml of FeEDTA solution (Sheat et al., 1959) and 0.25 ml of a minor element solution (Whittier and Steeves, 1960) were added per liter. The mineral nutrients



FIGS. 1–10. Early development of *Stromatopteris* gametophytes. 1) Spore with nucleus, equatorial longitudinal view. 2) Splitting of laesura in early germination, proximal view. 3) Cell beginning to bulge out of spore coat, equatorial longitudinal view; arrow indicates spore coat. 4) First cell division of gametophyte occuring with spore coat (arrow) still present, proximal view. 5) Two-celled gametophyte without spore coat. 6) Three-celled gametophyte. 7) Four-celled filamentous gametophyte. 8) Four-celled gametophyte after one oblique division. 9) Gametophyte with two oblique divisions at apical end and abortive rhizoid at basal end. 10) Six-celled gametophyte with two oblique divisions at apical end. Bar = 50 μm.

were supplemented with 0.5% glucose. The medium was solidified with 1% agar and its pH was 5.2 after autoclaving.

OBSERVATIONS

Stromatopteris spores are bean-shaped and monolete (Figs. 1, 2), and have an average length of 59 μm. The contents of the spores, especially the nucleus (Fig. 1), are easily observed because the spore coat is transparent. The nucleus is close to the distal wall in a central position along the longitudinal axis of the spore (Fig. 1). The remainder of the spore contains large numbers of small oil droplets, which stain with Sudan IV.

After the spores had been on the nutrient medium in the dark for three months, they began to germinate. No spores were observed to germinate in illuminated cultures.

The monolete laesura (scar) splits in the middle to initiate germination (Fig. 2). As the laesura ruptures to its ends, the cell within bulges out slightly (Fig. 3). Before the cell escapes from the spore coat, the first cell division occurs (Fig. 4). This division is parallel to the polar axis of the spore (Fig. 4). The cell wall forms perpendicular to the long axis of the spore and is displaced towards

one end of the spore. One of the resulting cells is somewhat larger than the other.

The cells of the two-celled filament enlarge and usually escape from the spore coat (Fig. 5). Neither of these cells differentiate into a rhizoid (Fig. 5). The second division in the young gametophyte is parallel to the first (Fig. 6) and a three-celled filament is formed. Often the cells are wider than long but some elongation may take place after they are formed. With some gametophytes, a third division occurs parallel to the first two and a four-celled filament is formed (Fig. 7). Usually, the filamentous or protonemal stage of these gametophytes is three or four cells in length.

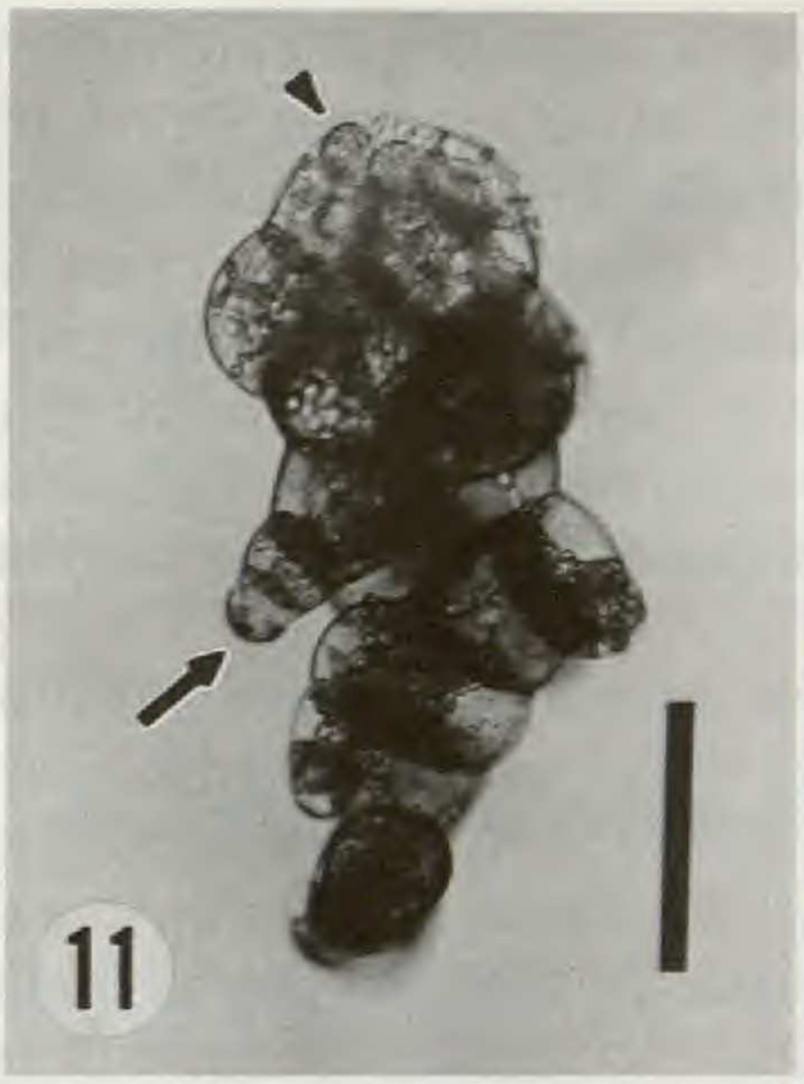
Once the filament reaches its maximum cell length, oblique cell divisions occur in a cell at one end of the filament (Figs. 8–10). After three or four oblique divisions, an apical cell with three cutting faces is established. The apical cell (Figs. 11, 12) initiates the axial three-dimensional growth of the gametophyte. The shift from filamentous to axial growth happens quickly with very few cell divisions. Rarely do these gametophytes remain two-dimensional beyond the second or third oblique division. Young gametophytes with apical cells and short, three-dimensional regions are more or less conical (Figs. 11, 13, 14). The activity of the apical cells forms the cylindrical portions of the older gametophytes.

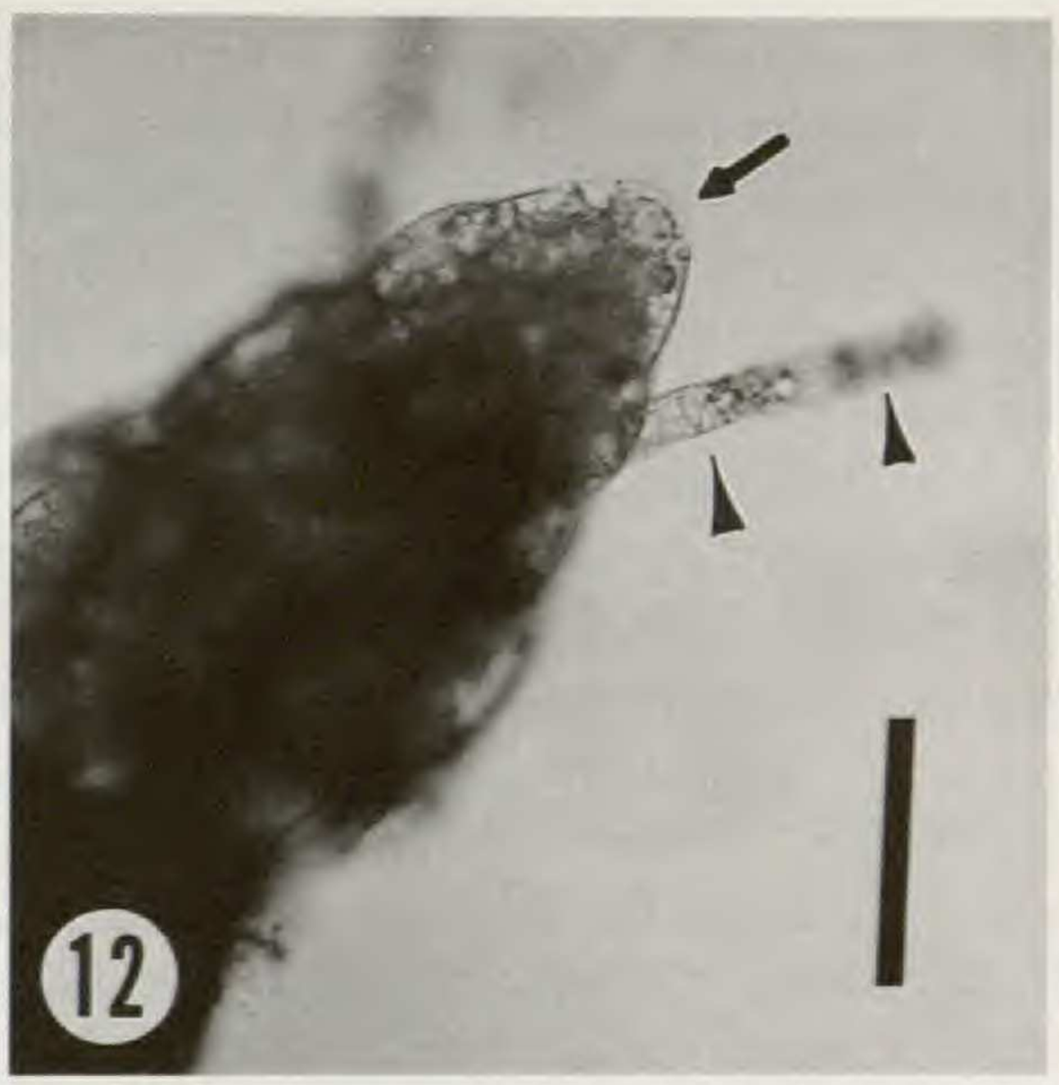
The oldest gametophytes obtained after a year in culture were narrow, cylindrical gametophytes with conical apices each topped by an apical cell (Fig. 12). However, the largest of these gametophytes were still small, being only 0.5 mm in length. Often the basal portions of the older gametophytes are dark brown to almost black from tanniferous materials in their cell walls (Figs. 12, 13). No gametangia have been found on any gametophytes grown to date in culture.

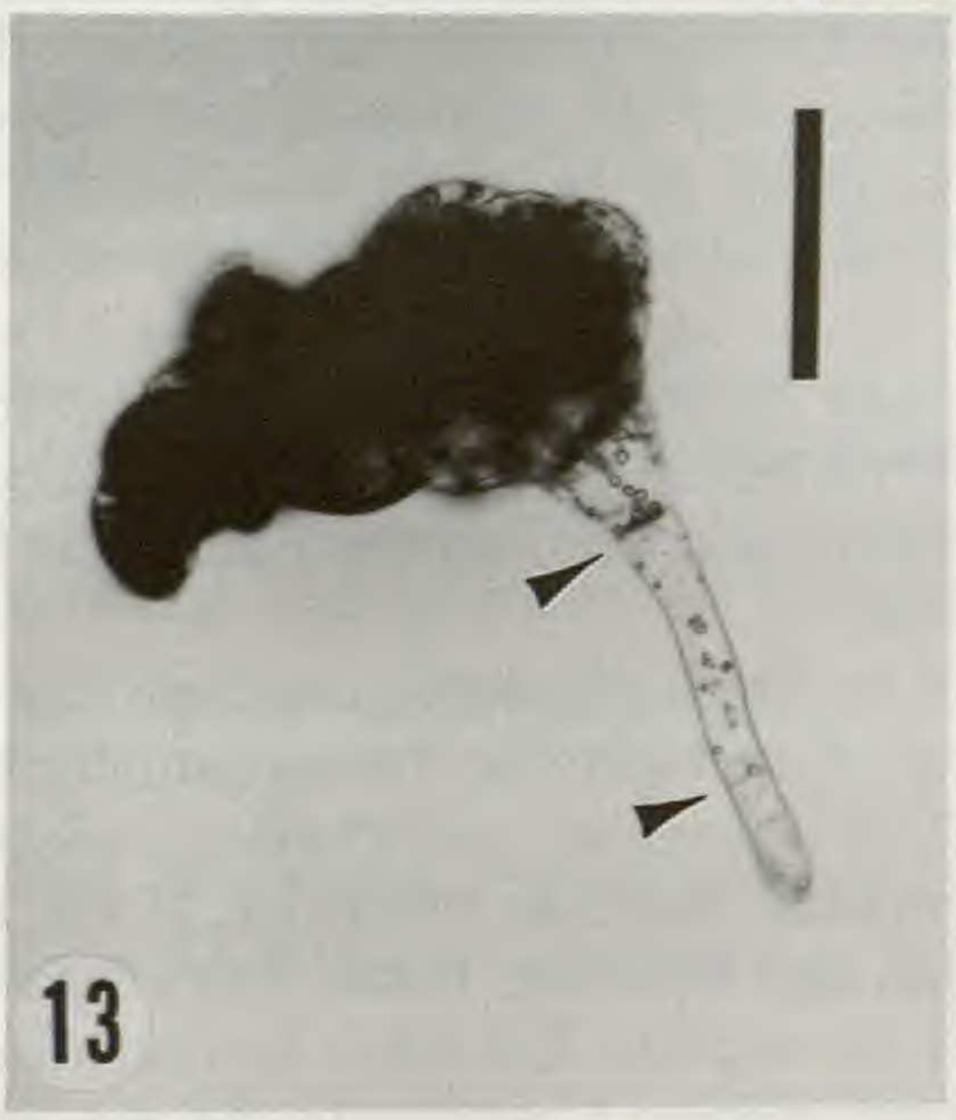
Mature rhizoids rarely form on the youngest gametophytes. Occasionally, after oblique divisions occur at the apical end, the basal cell of the filament will divide obliquely or almost perpendicularly to the original cell walls of the filament (Fig. 9). This forms a small cell on the side of the basal cell that undergoes minimal enlargement and no further cell divisions. This cell, which often contains more tanniferous materials in its wall than the walls of other prothallial cells, appears to be an abortive rhizoid, as sometimes observed on young *Psilotum* gametophytes (Whittier, 1975). Later gametophytes often form small cells with tanniferous walls on the sides of the thicker gametophyte regions (Figs. 11, 14). Usually, these cells do not elongate, possibly because the surface of the nutrient medium is wet. They also appear to be abortive rhizoids. On larger gametophytes one to a few elongate rhizoids usually develop. These are septate being composed of a uniseriate filament of three to four cells (Figs. 12–14).

Discussion and Conclusions

The germination of *Stromatopteris* spores occurs in the dark and is slow. Germination in darkness after several weeks or a few months is characteristic







FIGS. 11–14. Development of *Stromatopteris* gametophytes. 11) Small gametophyte with apical cell (arrowhead) and abortive rhizoid (arrow). 12) Apex of larger gametophyte with apical cell (arrow) and septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoids. 13) Small gametophyte with septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoids. 14) Small gametophyte with abortive rhizoid (arrow) and septate rhizoid; arrowheads indicate transverse cell walls of septate rhizoid. Bars = 100 μm.

of spores from species with mycorrhizal gametophytes (Whittier, 1981, 1998; Whittier and Braggins, 1994). The conditions for and timing of germination of spores of *Stromatopteris,* a leptosporangiate species, are similar to those for spores of eusporangiate species of ferns or other pteridophytes that have mycorrhizal gametophytes. It appears important for the spores of species with this type of gametophyte to germinate in the dark. Germination in nature would presumably occur after the spores have been covered by soil or opaque leaf litter. This should improve the chances that the young gametophytes would be infected with mycorrhizal fungi.

The early pattern of cell divisions for *Stromatopteris* gametophytes is as described for the Gleicheniaceae (Stokey, 1950; Nayar and Kaur, 1971). In this family the first division is parallel to the polar axis of the spore and succeeding divisions are parallel to the original division. A filament is formed perpendicular to the polar axis of the spore, the equatorial plane. This filament may become several cells in length before oblique divisions begin to occur (Stokey, 1950). This pattern of divisions is characteristic for the Gleicheniaceae and this type of early gametophyte development has been named the Gleichenia-type by Nayar and Kaur (1971).

There are differences in early gametophyte development between *Stromatopteris* and the photosynthetic representatives of the Gleicheniaceae. The photosynthetic gametophytes have an extended two-dimensional growth phase arising from the original filament. This two-dimensional growth pattern eventually forms the early cordate gametophyte. In *Stromatopteris,* there is a very abbreviated two-dimensional phase before the cylindrical growth is initiated. Also, the timing of rhizoid formation varies between the photosynthetic gametophytes and those of *Stromatopteris.* In the photosynthetic gametophytes, the rhizoid develops from the basal cell of the filament before the two-dimensional phase is initiated. Usually, this rhizoid forms directly from one cell of the two-celled filament (Stokey, 1950). However, in some cases a cell is formed on the side of the basal cell and this becomes the rhizoid (Stokey, 1950). In *Stromatopteris* neither cell of the two-celled filament ever becomes a mature rhizoid. Rarely, an abortive rhizoid will form on the side of the basal cell after the gametophyte is multicellular.

The lack of early rhizoid development on *Stromatopteris* gametophytes does not appear unusual for young mycorrhizal gametophytes. Young gametophytes of *Psilotum, Tmesipteris, Botrychium, Ophioglossum,* and *Phylloglossum* first form abortive rhizoids and later form normal rhizoids (Whittier, 1975, 1981; Whittier and Braggins, 1992, 1994; Melan and Whittier, 1989). The rhizoids of *Stromatopteris* are unusual in that they are septate. On other mycorrhizal gametophytes, the rhizoids are normally unicellular. Contrary to the report that *Psilotum* gametophytes have septate rhizoids (Bierhorst, 1953), 97% of the rhizoids on *Psilotum* gametophytes are unicellular and only 3% are bi- or tricellular (Whittier, 1986). Also, only a small number of the rhizoids on *Botrychium* gametophytes are multicellular and they form on the antheridial ridge (Whittier and Peterson, 1984). Septate rhizoids are rarely found on mycorrhizal gametophytes other than *Stromatopteris.*

There is no similarity in the early pattern of cell divisions of *Stromatopteris* gametophytes and those of the Psilotaceae, Lycopodiaceae, or Ophioglossaceae. In the Ophioglossaceae, the first division is perpendicular to the polar axis of the spore forming proximal and distal cells (Whittier, 1981). In the Lycopodiaceae, the angle of the first division is variable. If a small rhizoid cell is cut off, the first division is parallel to the polar axis and the second division is oblique (Bruchmann, 1910). However, if the first division divides the original cell into two more or less equal cells then it is oblique to the polar axis (Whittier and Braggins, 1992). The monolete, bean-shaped spores of the Psi-

lotaceae are very similar to those of *Stromatopteris,* as noted be Bierhorst (1971). However, the first division in *Psilotum* and *Tmesipteris* is perpendicular to the polar axis of these spores. The early development of *Stromatopteris* gametophytes is different from the other mycorrhizal gametophytes because the first two or three divisions are parallel to the polar axis of the spore and form a short filament along the equatorial plane of the spore. Gametophytes from the other three families are not filamentous after the second division. The two- and three-dimensional growth of gametophytes of the Psilotaceae, Ophioglossaceae, and Lycopodiaceae is established with fewer divisions than with *Stromatopteris.*

Nayar and Kaur (1971) recognized three germination patterns in the ferns: polar, equatorial, and amorphous. The amorphous type was characterized as having no polarity with regard to cell divisions or direction of growth. This type is restricted to primitive fern groups and it is what Nayar and Kaur (1971) expected in *Stromatopteris.* However, the early development of the gametophyte is equatorial and characteristic of the Gleicheniaceae. It is the later development that has been modified to produce the cylindrical gametophytes of *Stromatopteris* rather than the cordate prothalli characteristic of the photosynthetic gametophytes from the Gleicheniaceae. It appears that mycorrhizal gametophytes can be formed with both the polar and equatorial germination patterns. Whether the amorphous type is involved with any mycorrhizal gametophyte is unclear at this time. Even though there is great variation in the early development of these mycorrhizal gametophytes, germination of the spores in the dark is consistent for all of them.

Acknowledgments

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